General Labjournal

25.06.2014
Grow on shakeflasks the 25 samples that we requested from the iGEM HQ. The biobrick numbers of the requested samples: K1172305, K1172306, K1172401, K1172403, K1172404, K917013, K917014, K1127006, K1172303, K1172304, K1179019, K917012, K917009, K917006, K917003, K1172405, K1172501, K1172502, K1172503, K1172504, K1172505, K1172507, K540000, K342003, K540001.

26.06.2014
Miniprep + Glycerol stock of the 25 samples that we requested from the iGEM HQ.

07.07.2014
Prepare solutions for competent cells preparation (MgCl2 and CaCl2)

08.07.2014
Prepare all the sequencing mixtures. The parts to be sequenced were: K1172306, K1172403, K1172404, K1172303, K917009, K917006, K917003, K1172502, K540000 and K342003.

15.07.2014
- Made competent cells of C43(DE3) and BL21(DE3) for transformation
- Cultivate the transformed constructs from 14.07.2014 in LB medium for miniprep
    - Also cultivate mKate (amp), eGFP (amp) and Rhamnose promoter - Bba_K914003 (cam)
    - And the strain AYCE189 from Lu lab is also cultivated (for a glycerolstock)

16.07.2014
- Miniprepped the samples cultivated on 15.07.2014, except for the strain AYCE189.
- Measured the concentration of the isolated DNA with nanodrop:
    - PAYC002 122.8 ng/ul
    - rr12y(rii)g 52.6 ng/ul
    - PAYC003 187.0 ng/ul
    - rrjt12(11)g 32.3 ng/ul
    - PAYC005 27.8 ng/ul
    - PAYC008 22.3 ng/ul
    - PAYC006 37.0 ng/ul
    - PAYC007 22.3 ng/ul
    - I5023 22.5 ng/ul
    - C640 14.3 ng/ul
    - mKate 39.2 ng/ul
    - eGFP 165.1 ng/ul
    - Rhamnose 47.7 ng/ul
- Cultivated the samples again in shakeflasks.
- Made glycerolstocks of all the cultivated samples from 15.07.2014.

Test the competency of C43(DE3)
- 30 ul competent C43 cells + 100 ng pUC19 (AmpR)(190 ng/ul), plated in duplo on Cam (neg. control), Amp (pos. control) LB plates and on LB plate without antibiotics

- 30 ul competent C43 cells plated in duplo on Cam (neg. control), Amp (neg. control) LB plates and on LB plate without antibiotics (pos. control)

17.07.2014
Results of the plates for positive and negative control of C43

- C43 + pUC19(AmpR) + Amp plates — Much growth
- C43 + pUC19(AmpR) + Cam plates — No growth
- C43 + pUC19(AmpR) + without antibiotic — Much growth
- 2x diluted C43 + pUC19(AmpR) + Amp plates — Single colonies
- 2x diluted C43 + pUC19(AmpR) + Cam plates — No growth
- 2x diluted C43 + pUC19(AmpR) + without antibiotic — Much growth
- C43 + no plasmid + Amp plates — No growth
- C43 + no plasmid + Kan plates — No growth
- C43 + no plasmid + Cam plates — No growth

We decided to not dilute the competent cells for the future experiments.
CFU for pUC19(AmpR): 1120 colonies on the plate.

Test the competency of BL21(DE3)

- 30 ul competent BL21 cells + 100 ng pUC19 (AmpR)(190 ng/ul), plated in duplo on Cam (neg. control), Amp (pos. control) LB plates and on LB plate without antibiotics
- 30 ul competent BL21 cells plated in duplo on Cam (neg. control), Amp (neg. control) LB plates and on LB plate without antibiotics (pos. control)
- 2x diluted 30 ul competent BL21 cells + 100 ng pUC19 (AmpR)(190 ng/ul) plated in duplo on Amp (pos. control) LB plates

18.07.2014
Results of the plates for positive and negative control of BL21(DE3)

- BL21 + pUC19(AmpR) + Amp plates — Much growth
- BL21 + pUC19(AmpR) + Cam plates — No growth
- BL21 + pUC19(AmpR) + without antibiotic — Much growth
- 2x diluted BL21 + pUC19(AmpR) + Amp plates — Single colonies
- BL21 + no plasmid + Amp plates — No growth
- BL21 + no plasmid + without antibiotic — Much growth
- BL21 + no plasmid + Cam plates — No growth

22.07.2014
Prepared antibiotics, 500ul in each eppendorf cup. Saved in 'Antibiotics' box in the freezer with other iGEM materials. 1000x stock solutions.

- Chloramphenicol diluted in EtOH
  34 mg/ml in EtOH -> 0,391 gram diluted in 11,5 ml EtOH
- Kanamycin diluted in H2O
  10 mg/ml in H2O -> 0,095 gram diluted in 9,5 ml H2O
- Ampicillin diluted in H2O
  100 mg/ml in H2O -> 0,92 gram diluted in 9,2 ml H2O

Pre-culture DH5-alpha has been prepared, 100 mL LB medium is cultivated and placed in the shaker. Overnight at 37 degrees and 180 rpm.

23.07.2014

Made competent cells of DH5a and saved in the freezer (-80).

28.07.14
The samples asked from Exeter iGEM team were prepared and sent to them. The samples requested are:
- BBa_K1022115 , Kanamycin resistant
- BBa_K1022105 , Chloramphenicol resistant
- BBa_K112808 , Ampicillin resistant

Transformation of pUC19 in BL21 and DH5a as a control

- 30ul + 100ng pUC19 (190 ng/ul)
  - Plated 50ul and 100 ul on Cam, Amp and plates without antibiotics
- 30ul competent cells
  - Plated 50ul and 100 ul on Cam, Amp and plates without antibiotics

29.07.2014
Cristy and Anne
Results of the DH5a and BL21 plates:

- BL21 + pUC19(AmpR) + Amp plates — Much growth, big single colonies
- BL21 + pUC19(AmpR) + Cam plates — No growth
- BL21 + pUC19(AmpR) + without antibiotic — Much growth
- BL21 + no plasmid + Amp plates — No growth
- BL21 + no plasmid + without antibiotic — Much growth
- BL21 + no plasmid + Cam plates — No growth
- DH5a + pUC19(AmpR) + Amp plates — Much growth, little single colonies
- DH5a + pUC19(AmpR) + Cam plates — No growth
- DH5a + pUC19(AmpR) + without antibiotic — Much growth
- DH5a + no plasmid + Amp plates — No growth
- DH5a + no plasmid + without antibiotic — Much growth
- DH5a + no plasmid + Cam plates — No growth

Tomek and Esra: making trace elements solution 2 attempts

29-7-2014

Making the Trace Elements Solution

Chemical in paper - 1	Chemical in our buffer -2	g/mol - 1	g/mol -2	Weight (gram) in article	Weight (gram) in our buffer	Company	CAS number	Cupboard number
Na2EDTA	Na2EDTA.2H2O	336,2	372,24	2,26	2,517	Sigma-Aldrich1	6381-92-6	E25
MgSO4.7H2O		246,47		24,89	24,89	JT Baker2	10034-99-8	M03
MnSO4.4H2O	MnSO4.H2O	169,02	223,08	0,029	0,022	Sigma-Aldrich1	10034-96-5	M07
NaCl		58,44		0,058	0,058	JT Baker2	7647-14-5	S17
FeCl2	FeCl2.4H2O	126,73	198,81	0,068	0,107	Tu Delft afd. FD-LMS	231-729-4	I04
CoCl2	CoCl2.6H2O	129,81	237,93	0,065	0,119	JT Baker2		C08
ZnSO4.7H2O		287,54		0,029	0,029	Merck		Z03
CuSO4.5H2O		249,68		0,005	0,005	JT Baker2	7758-99-8	C12
H3BO3		61,83		0,35	0,35	USB	10043-35-3	B11
Na2MoO4	Na2MoO4.2H2O	205,91	241,95	0,08	0,094	Sigma-Aldrich1	10102-40-6	S24
NiCl2.6H2O		237,7		0,119	0,119	Aldrich	7791-20-0	N41
Na2SeO4		188,9		0,028	0,028	Sigma		S55

- FeCl2.4H2O is diluted in 22,5 mL HCL
- Adding order: EDTA + 800 mL H2O -> FeCl2.4H2O + 25 mL 6 M HCl -> MgSO4.7H2O -> etc
- Precipitation after adding MnSO4.4H2O - > o/n on a stirrir - > precipitation still there (30-7-2014) -> failed..

30.07.2014
Esra
Making the M4 minimal medium Trace Elements Solution (third attempt) + buffer (exact contents will be updated)
-Changed adding order
-Adding order 1- EDTA, 2- Mg, 3-Mn, 4-NaCl, 6-CoCl etc.
-Added number 5- (FeCl diluted in 22.5 mL HCl) at the end!!
-All added except FeCl (in HCl) -> no precipitations!! All is diluted well!

Inoculated the transformed iGEM registry constructs

Resistance	Transformation	Grown in (ml LB):
Cam	DH5a + k823017	5ml & 10 ml
Cam	DH5a + k808000	5ml & 10 ml
Kan	DH5a + I20260	5ml & 10 ml
Cam	DH5a + 80017	5ml & 10 ml
Amp	DH5a + J231100	5ml & 10 ml
Amp	DH5a + pUC19	5ml & 10 ml

20.08.2014
Janna
Made medium M4 with different carbon sources.
1. 400 ml 40 mM D/L-lactate M4
   342 ml MilliQ
   10 ml Buffer 40 x
   4 ml 0.1 M $CaCl_2$
   40 ml 0.4 M D/L-lactate
   4 ml Trace elements 100 x
2. 400 ml 40 mM glycerol M4
   342 ml MilliQ
   10 ml Buffer 40 x
   4 ml 0.1 M $CaCl_2$
   40 ml 0.4 M Glycerol
   4 ml Trace elements 100 x
3. 400 ml 40 mM glucose M4
   342 ml MilliQ
   10 ml Buffer 40 x
   4 ml 0.1 M $CaCl_2$
   40 ml 0.4 M Glucose
   4 ml Trace elements 100 x

21.08.2014
Janna

Tested competent cells BL21 culture 1, BL21 culture 2 and C43. I used pUC19 as test DNA (ampR). Made the following combinations:
1. 30 µl BL21.1 with 1 µl MilliQ
2. 30 µl BL21.1 with 1 µl pUC19
3. 30 µl BL21.2 with 1 µl MilliQ
4. 30 µl BL21.2 with 1 µl pUC19
5. 30 µl C43 with 1 µl MilliQ
6. 30 µl C43 with 1 µl pUC19

There were no colonies on the plates, so the cells are not competent.

Cristy: Made competent cells of CsgB (approximately 30 eppendorfs containing 100ul competent cells). OD600: 0,567 and 0,589

21.08.2014
Joan
Prepare samples for Melbourne iGEM team:

- pET23b - Ulp1-His6 (AmpR)
- BBa_K1022107:pcI-Ulp in pSB1C3 col2
- BBa_K1022113:pBAD-Ulp-TT in pSB1C3 col2

26.08.2014
Chloramphenicol diluted in EtOH
34 mg/ml in EtOH -> 0,3912 gram diluted in 11,5ml EtOH. Divided in aliquots of 500 ul. Refilled stock with 22 new cups.

Janna
Making M4 with D/L-lactate. Same protocol as 20/8, only filter sterilized the whole bottle after making it.

28.08.2014
Janna
Transformation of pUC19 in C43+ET20 as test
Made the following transformations:
0.5 ul pUC19 plasmid (concentration of 100.2 ng/ul) added to 30 ul of C43+ET20 competent cells
1 ul MilliQ added to 30 ul of C43+ET20 competent cells as a negative control

Followed the transformation in home-made competent cells protocol and made six plates with each 100 ul:
pUC19 in C43+ET20 (ampR and camR):
Ampicillin - some growth
Chloramphenicol - some growth
Amp and Cam - lots of growth
MQ in C43+ET20 (camR):
Ampicillin - no growth
Chloramphenicol - some growth
Amp and Cam - no growth

This means the cells are competent, but the efficiency is low.